

#4923 Store at -20°C

Non-phospho-4E-BP1 (Thr46) (87D12) Rabbit mAb



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Entrez-Gene ID #1978, 1979, 8637
UniProt ID #Q13541 (H1), Q13542 (H2), O60516 (H3)

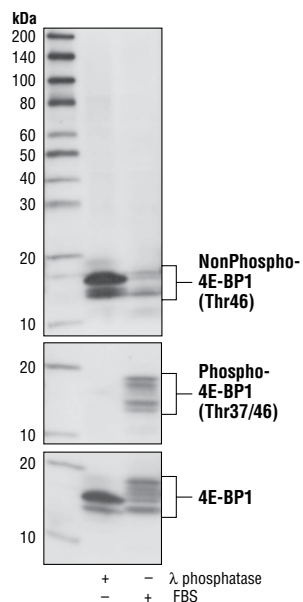
Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IF-IC, F Endogenous	H, M, R, Mk	15-20 kDa	Rabbit IgG**

Background: Translation repressor protein 4E-BP1 (also known as PHAS-1) inhibits cap-dependent translation by binding to the eIF4E translation initiation factor. Hyperphosphorylation of 4E-BP1 disrupts this interaction and results in activation of cap-dependent translation (1). Both the PI3 kinase/Akt pathway and FRAP/mTOR kinase regulate 4E-BP1 activity (2,3). Multiple 4E-BP1 residues are phosphorylated *in vivo* (4). While phosphorylation by FRAP/mTOR on Thr37 and Thr46 does not prevent the binding of 4E-BP1 to eIF4E, it is thought to prime 4E-BP1 for subsequent phosphorylation at Ser65 and Thr70 (5).

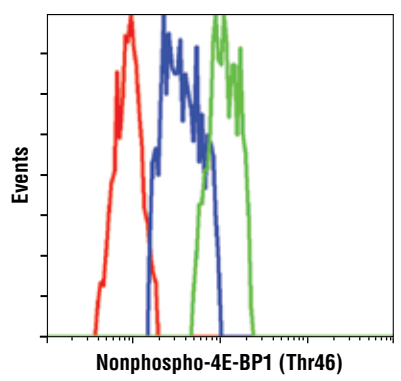
Specificity/Sensitivity: Non-phospho-4E-BP1 (Thr46) (87D12) Rabbit mAb detects endogenous levels of 4E-BP1 only when dephosphorylated at Thr46. The antibody cross-reacts with 4E-BP2 and 4E-BP3 dephosphorylated at equivalent sites.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Thr46 of human 4E-BP1.

- Background References:**
- (1) Pause, A. et al. (1994) *Nature* 371, 762–767.
 - (2) Brunn, G.J. et al. (1997) *Science* 277, 99–101.
 - (3) Gingras, A.C. et al. (1998) *Genes Dev.* 12, 502–513.
 - (4) Fadden, P. et al. (1997) *J. Biol. Chem.* 272, 10240–10247.
 - (5) Gingras, A.C. et al. (1999) *Genes Dev.* 13, 1422–1437.



Western blot analysis of extracts from COS cells, treated with λ phosphatase or 20% FBS as indicated, using Non-phospho-4E-BP1 (Thr46) (87D12) Rabbit mAb (upper), Phospho-4E-BP1 (Thr37/46) Antibody #9459 (middle) and 4E-BP1 Antibody #9452 (lower).



Flow cytometric analysis of COS cells, untreated (blue) or λ phosphatase treated (green), using Non-phospho-4E-BP1 (Thr46) (87D12) Rabbit mAb compared to a nonspecific negative control antibody (red).

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunofluorescence (IF-IC)	1:200
Flow Cytometry	1:200

For application specific protocols please see the web page for this product at www.cellsignal.com.

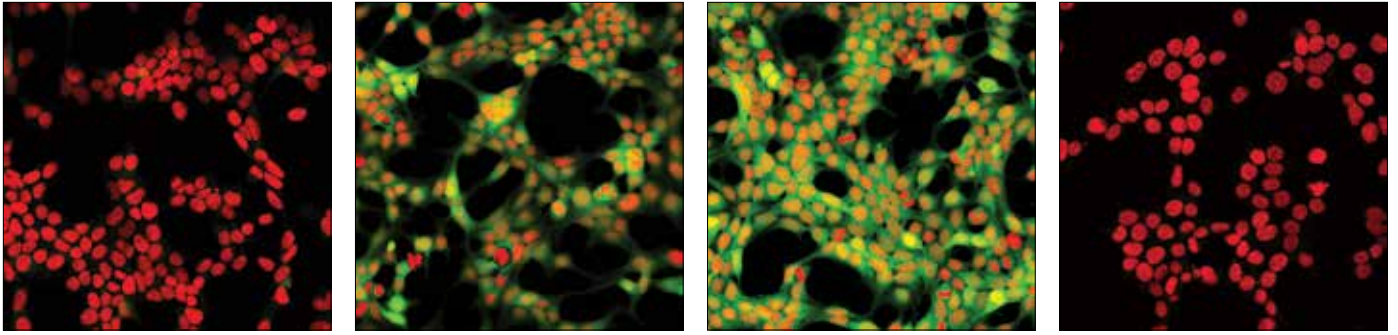
Please visit www.cellsignal.com for a complete listing of recommended companion products.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

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Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
 Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Confocal immunofluorescent analysis of 293 shRNA Scramble cells either serum starved (far left); serum starved and treated with U0126 #9903 (10 μ M, 2 hr), LY294002 #9901 (50 μ M, 2 hr), and Rapamycin #9904 (10 nM, 2 hr) (center, left); serum starved and treated with λ phosphatase (10,000 U/mL, 2 hr) (center, right); and 293 shRNA 4E-BP1/2 KO treated with U0126 #9903 (10 μ M, 2 hr), LY294002 #9901 (50 μ M, 2 hr), and Rapamycin #9904 (10 nM, 2 hr) (far right), using Non-phospho-4E-BP1 (Thr46) (87D12) Rabbit mAb (green). Red = Propidium Iodide (PI)/RNase Staining Solution.